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(54) Title: RENAL REGULATORY ELEMENTS AND METHODS OF USE THEREOF

(57) Abstract: Disclosed are *cis*-acting regulatory elements from a KIM-1 gene. The elements can be used to direct the expression of operably linked sequences in renal tissue.

Renal Regulatory Elements and Methods of Use Thereof

STATEMENT OF GOVERNMENT INTEREST

This invention was made with federal government support under grant #DK 39773. The United States government has certain rights in the invention.

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FIELD OF THE INVENTION

The invention relates generally to nucleic acids and more particularly to nucleic acids which can be used to direct expression in renal tissue of operably linked sequences.

BACKGROUND OF THE INVENTION

Significant interruption in kidney function in an individual can lead to incapacitation or even death. Disease or injury can impair kidney function. An example of an injury that can damage kidneys is ischemic injury. In this type of injury, kidney tissue is damaged because of oxygen deprivation occurring as a result of interruption of blood flow to the kidneys.

Certain agents involved in repair of diseased or damaged kidney tissue, and mechanisms in which the repair takes place, have been described. Mechanisms include induction of gene expression and recruitment of growth factors to the affected kidney tissue. Cell death and cellular proliferation are also associated with repair or kidney tissue.

Agents implicated in kidney tissue repair include polypeptides, *e.g.*, growth factors such as insulin growth factor (IGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and the endothelial cell adhesion molecule ICAM-1.

Recently, the polypeptide kidney-injury molecule (KIM-1) has been described. The expression of KIM-1 is increased in injured kidney tissue. The rat and human forms of this protein have been characterized. The KIM-1 cDNA sequence reveals that the KIM-1 protein is a type 1 membrane protein that contains a novel six-cysteine immunoglobulin-like domain and a mucin domain. The KIM-1 protein is a member of the immunoglobulin gene superfamily and most closely resembles mucosal addressin cell adhesion molecule 1 (MAdCAM-1).

SUMMARY OF THE INVENTION

It has been discovered that nucleic acid sequences in the vicinity of the human KIM-1 gene can be used to express linked sequences in renal tissue. Accordingly, the invention provides a *cis*-acting regulatory element useful for, *inter alia*, directing expression of an

operatively linked sequence, *e.g.*, a gene, in a mammal. The *cis*-acting KIM-1 regulatory sequence can also be used to identify *trans*-acting factors that mediate the response of the kidney to damaged or diseased tissue.

The invention provides an isolated nucleic acid that includes a *cis*-acting KIM-1
5 derived regulatory sequence. The nucleic acid can be, *e.g.*, a nucleic acid sequence that includes the nucleic acid sequence of SEQ ID NOs:1, 2 or 3. The nucleic acid includes at least 5 contiguous nucleotides from a sequence that hybridizes to SEQ ID NOs: 1, 2, or 3, or sequences complementary to SEQ ID NOs: 1, 2, or 3. For example the regulatory sequence can include between 5 and 35 contiguous nucleotides from SEQ ID NO:3, or sequences
10 complementary to such portions of SEQ ID NO:3.

In some embodiments, a *cis*-acting KIM-1 regulatory sequence according to the invention includes a portion of SEQ ID NOs:1, 2 or 3 sufficient to regulate kidney tissue-specific transcription of an operably linked sequence, *e.g.*, an operably linked polypeptide-encoding sequence. A *cis*-acting KIM-1 regulatory sequence according to the invention can
15 include a portion of SEQ ID NOs:1, 2 or 3 is sufficient to regulate kidney tissue-specific transcription following cellular injury *e.g.*, anoxia or exposure to reactive oxygen species ("ROS"), or in a cell present in a confluent population of cells.

The invention also provides a *cis*-acting KIM-1 regulatory sequence operably linked to a sequence encoding a KIM-1 antisense nucleic acid. The *cis*-acting KIM-1 regulatory
20 sequence can be operably linked to at least one polypeptide-encoding sequence and regulates renal tissue-specific transcription of the polypeptide-encoding sequence. For example, the polypeptide-encoding sequence may encode a KIM-1 polypeptide (*e.g.*, a human KIM-1 polypeptide), or a non-KIM-1 polypeptide. This polypeptide can be, *e.g.*, a cell survival-promoting factor, a cell growth-promoting factor, a wound-healing factor, an anti-fibrotic
25 factor, an apoptosis-inhibiting factor, an anti-inflammatory factor, a terminal differentiation-promoting factor, a cell growth-inhibiting factor, an intravascular-volume restoration factor, a chelating agent, an alkylating agent, an angiotensin-converting enzyme-inhibiting factor, erythropoietin, a cytokine, a receptor, an anticoagulant, an enzyme, a hormone, an antibody, or a renal structural protein.

30 A *cis*-acting KIM-1 regulatory sequence according to the invention may be linked to, *e.g.*, nucleic acid sequences encoding insulin growth factor (IGF), an epidermal growth factor (EGF), a fibroblast growth factor (FGF), a transforming growth factor beta (TGF β) Type II receptor, a hepatocyte growth factor (HGF), or an endothelial cell adhesion molecule ICAM-1.

The invention also provides a vector that includes a nucleic acid comprising a *cis*-acting KIM-1 regulatory sequence and cells containing these nucleic acids and vectors. The cell can be prokaryotic or eukaryotic. The cell can be, *e.g.*, a metazoan organism or a unicellular organism, and can include, *e.g.*, a fungal cell, yeast cell (such as *Saccharomyces*,
5 *Schizosaccharomyces*, or *Candida* spp.), or a mammalian cell, *e.g.*, a human, canine, bovine, porcine, feline, or rodent cell, or a non-human mammalian embryonic blastocyst cell.

The invention also provides a transgenic non-human mammal, *e.g.*, a mouse, rat, goat, pig, cow, or sheep, containing an isolated *cis*-acting KIM1 regulatory sequence. The transgenic animal can be produced, *e.g.*, by intrauterine implantation of a blastocyte cell
10 containing a *cis*-acting KIM-1 regulatory sequence. The invention also includes one or more progeny of the transgenic non-human mammal DNA, wherein the progeny comprises the *cis*-acting DNA, or a fragment thereof.

The invention also provides a method of directing expression of a polypeptide. The method includes providing a cell, *e.g.*, a renal cell, that includes an isolated *cis*-acting KIM-1
15 regulatory sequence operably linked to sequence encoding a polypeptide of interest, culturing the cell under conditions that allow for the expression of the polypeptide and expressing the polypeptide-encoding sequence.

The invention also includes a method of increasing transcription of a polypeptide-encoding sequence in tissue. The method includes providing a cell in the tissue that includes a
20 *cis*-acting KIM-1 regulatory sequence linked to the polypeptide-encoding sequence and culturing the cell under conditions that allow for the transcription of the polypeptide-encoding sequence. The polypeptide-encoding sequence is then expressed, resulting in transcription of the polypeptide-encoding sequence in the tissue.

The invention also includes a method for identifying a test compound that modulates
25 expression from a *cis*-acting KIM-1 derived regulatory sequence. The test compound can be contacted with a reporter construct that includes a reporter gene operably linked to an isolated *cis*-acting KIM-1 regulatory sequence. The level of expression of the reporter gene in the tissue is detected, *e.g.*, measured. A change in the level of expression in the presence of the test compound relative to the level of expression in the absence of the test compound indicates
30 that the test compound modulates the activity of the KIM promoter.

The invention also provides a method for delivering a therapeutic polypeptide to renal tissue of a subject. The method includes providing in the renal tissue a cell that includes a *cis*-acting KIM-1 regulatory sequence operably linked to a therapeutic polypeptide, and culturing

the cell under conditions that allow for the expression of the polypeptide. The polypeptide-encoding sequence is expressed, thereby delivering the therapeutic polypeptide to the renal tissue of the subject.

5 The invention also includes a method for treating or preventing renal tissue injury. The method includes providing a cell that includes *cis*-acting KIM-1 regulatory sequence operably linked to a polypeptide coding sequence, and culturing the cell under conditions that allow for the expression of a therapeutic polypeptide-encoding sequence. The therapeutic polypeptide-encoding sequence is expressed, and the expressed polypeptide contacts the renal tissue, thereby treating or preventing renal tissue injury.

10 The invention also includes a method for increasing transcription of a nucleic acid in a subject by administering to the subject a *cis*-acting KIM-1 regulatory sequence operably linked to the nucleic acid and allowing for expression of the operably linked nucleic acid.

The invention also provides a method for treating or preventing renal tissue injury in a subject by administering to a subject in need thereof a *cis*-acting KIM-1 regulatory sequence
15 operably linked to a sequence encoding a therapeutic polypeptide, in an amount sufficient to treat or prevent renal tissue injury in the subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can
20 be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

25 Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-H are schematic representations of a nucleic acid sequence that includes a *cis*-acting KIM-1 regulatory sequence (SEQ ID NO:1).

30 FIGS. 2A-D are schematic representations of sequences from the 5' region of the human KIM-1 gene.

FIG. 3 is a bar graph showing relative expression of luciferase in COS monkey kidney cells of various reporter constructs containing sequences from the 5' region of the human KIM-1 gene

FIG. 4 is a bar graph showing relative expression of luciferase in LLCPK cells of various reporter constructs containing sequences from the 5' region of the human KIM-1 gene.

FIG. 5 is a bar graph showing relative expression of luciferase in MDCK cells of various reporter constructs containing sequences from the 5' region of the human KIM-1 gene.

FIG. 6 is a bar graph showing inducibility of sequences linked to a KIM-1 regulatory sequence in HK2 cells after exposure to reactive oxygen species (ROS) or anoxia.

FIG. 7 is a bar graph showing relative expression of luciferase in MDCK cells at various timepoints after transfection with reporter constructs containing sequences from the 5' region of the human KIM-1 gene.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a *cis*-acting regulatory element useful for, *inter alia*, directing expression of an operatively linked sequence, *e.g.*, a gene, in a mammal. This *cis*-acting regulatory sequence from KIM-1 can also be used to identify *trans*-acting factors that mediate the response of the kidney to damaged or diseased tissue.

Sequence Identifier Numbers (SEQ ID NOs)

Sequence identifier numbers used herein include the following:

SEQ ID NO:1 corresponds to the nucleotide sequence of an 8933 bp human genomic DNA from the 5' region of KIM-1 gene and is disclosed in FIGS 1A-H. This fragment is present as a BamHI-BamHI insert in the BamHI site of the EMBL3 phage vector. The construct is named MZ007.

SEQ ID NO:2 corresponds to the nucleotide sequence of a 4.8 kb KpnI-KpnI fragment encompassing nucleotides 3796 to 8612 of the human KIM-1 insert in MZ007.

SEQ ID NO:3 corresponds to the nucleotide sequence of a 1.3 kb EcoRI-KpnI fragment encompassing nucleotides 7322-8612 of human KIM-1 insert in MZ007.

SEQ ID NO:4 corresponds to the nucleotide sequence of a 0.5 kb SacII-KpnI fragment encompassing nucleotides 8110-8612 of human KIM-1 insert in MZ007.

***Cis*-acting KIM-1 derived regulatory sequences**

Included in the invention is an isolated DNA that includes a *cis*-acting KIM-1 derived regulatory sequence. The term "isolated" refers to molecules separated from other DNA or RNA molecules, present in the natural source of the regulatory sequence. The term also refers
5 to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. An isolated nucleic acid includes nucleic acid fragments that are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to polypeptides that are isolated from
10 other cellular proteins, and the term is meant to encompass both purified and recombinant polypeptides.

A *cis*-acting KIM-1 derived regulatory sequence, also termed herein "*cis*-acting regulatory element", "regulatory element", or "regulatory sequence", includes nucleic acid sequence elements derived from sequences in the vicinity of a mammalian KIM-1 gene that are
15 capable of modulating transcription from a basic promoter, as well as enhancers or silencers. The terms "promoter" and "regulatory element" further encompass "tissue specific" promoters and regulatory elements, *i.e.*, promoters and regulatory elements which bring about expression of an operably linked DNA sequence preferentially in specific cells (*e.g.*, cells of a renal tissue). Gene expression occurs preferentially in a specific cell if expression in this cell type is
20 significantly higher than expression in other cell types. The terms "promoter" and "regulatory element" also encompass so-called "leaky" promoters and "regulatory elements", which regulate expression of a selected DNA primarily in one tissue, but cause expression in other tissues as well. The terms "promoter" and "regulatory element" also encompass non-tissue specific promoters and regulatory elements, *i.e.*, promoters and regulatory elements which are
25 active in most cell types.

A promoter or regulatory element can be a constitutive promoter or regulatory element, *i.e.*, a promoter or regulatory element which constitutively regulates transcription, or it can be a promoter or regulatory element which is inducible, *i.e.*, a promoter or regulatory element which is active primarily in response to a stimulus. A stimulus can be, *e.g.*, a physical
30 stimulus, such as injury (*e.g.*, ischemia), and/or a molecule, such as a hormone, a cytokine, a heavy metal, phorbol esters, cyclic AMP (cAMP), or retinoic acid.

The term "enhancer", also referred to herein as "enhancer element", includes regulatory elements capable of increasing, stimulating, or enhancing transcription from a basic promoter.

The term "silencer", also referred to herein as "silencer element" is intended to include regulatory elements capable of decreasing, inhibiting, or repressing transcription from a basic promoter. The terms "promoter" and "regulatory element" further encompass "tissue specific" promoters and regulatory elements, *i.e.*, promoters and regulatory elements which effect
5 expression of the selected DNA sequence preferentially in specific cells (*e.g.*, cells of a specific tissue). Gene expression occurs preferentially in a specific cell if expression in this cell type is significantly higher than expression in other cell types.

In some embodiments, one or more copies of a *cis*-acting regulatory element is present within a transcribed region of a KIM-1 gene. In other embodiments, the *cis*-acting regulatory
10 element is located 5' to the transcriptional start site of a KIM-1 gene.

In some embodiments, a *cis*-acting regulatory sequence is operably linked sequence to a promoter that is not derived from the native KIM-1 gene, and to a heterologous sequence, such as a polypeptide-encoding sequence. In other embodiments, the *cis*-acting regulatory sequence is operably linked to a KIM-1 promoter sequence and a heterologous sequence, such
15 as a polypeptide-encoding sequence.

In some embodiments, the *cis*-acting regulatory sequence includes the 1.3 kb sequence of SEQ ID NO:3, *e.g.*, the regulatory sequence can include the nucleotides of SEQ ID NO:1 and SEQ ID NO:2, as well as SEQ ID NO:3. In other embodiments, the *cis*-acting regulatory sequence includes a portion of SEQ ID NO:3 that is sufficient to confer renal tissue expression
20 of an operably linked sequence which otherwise would not be expressed in renal tissue. For example, the *cis*-acting regulatory sequence may include at least 5, 10, 15, 20, 25, 30, 35, 50, 100, 125, or 150 contiguous nucleotides from SEQ ID NO:3, or sequences complementary to SEQ ID NO:3. Thus, if desired, sequences responsible for conferring renal cell-specific expression in the sequence of SEQ ID NO:3 can be localized more precisely. Localization can
25 be performed using methods well-known in the art, *e.g.*, by constructing plasmids containing successively smaller portions of the 1.3 kb fragment of SEQ ID NO:3 placed upstream of a luciferase reporter gene in a construct such as pGL3 Basic (Promega Corporation, Madison, WI), or in any of the many reporter genes known in the art. The construct is then transfected into kidney cells. Suitable kidney cells include, *e.g.*, COS, LLC/PK1, and MDCK cells.
30 Increased expression of the reporter gene in kidney cells compared to the expression of the starting construct alone indicates that the smaller test fragment of the 1.3 kb DNA allows for renal tissue expression. Higher expression of the test fragment in renal tissue as compared to

other cell types (*i.e.*, fibroblast cells or non-smooth muscle cells) indicates that the DNA directs polypeptide expression in a renal tissue-specific manner.

Similarly, in other embodiments, the *cis*-acting regulatory sequence is sufficient to confer both inducible (*e.g.*, upon exposure to a stimulus) and tissue-specific expression in injured renal tissue. For example, the sequence can include at least the portion or portions of SEQ ID NO: 2 that are necessary and sufficient for such expression. Such portion(s) can be localized routinely as described above.

KIM-1 *cis*-sequences according to the invention can be used to direct expression of linked sequences following injury or under various conditions. For example, a nucleotide sequence that includes SEQ ID NO:2 (the 4.8 kb KpnI-KpnI fragment) can be used to direct expression of a linked polypeptide in cells that have been subjected to injury using a reactive oxygen species ("ROS"), or subjected to injury because of anoxia. A nucleotide sequence that includes at least the relevant portion or portions of SEQ ID NO:2 can also be used to direct expression of a sequence of interest in confluent cells.

In another embodiment, the isolated nucleic acid includes a *cis*-acting KIM-1 derived regulatory sequence that has been modified, *e.g.*, by adding, deleting, or substituting one or more nucleic acid residues. Such modifications can modulate the regulatory or transcriptional activity of the regulatory element. For example, a modification can increase or decrease the activity of a promoter or regulatory element. A modification can also affect the tissue specificity or inducibility of a promoter or regulatory element.

Desired modifications of a regulatory element according to the present invention can be performed according to methods well known in the art, such as by mutagenesis. The activity of the modified promoter or regulatory element can then be tested, using the herein described methods for assaying the *cis*-acting activity of a KIM-1 regulatory sequence.

In some embodiments, the regulatory sequence is inducible. As used herein, "inducible" means that the regulatory sequence affects expression of a linked sequence in response to a stimulus. The stimulus can be physical, *e.g.*, stress, such as heat shock, anoxia, or pressure, or chemical. Examples of chemical stimuli include, *e.g.*, a hormone, a cytokine, a heavy metal, phorbol esters, cyclic AMP (cAMP), or retinoic acid. In preferred embodiments, the regulatory sequence is inducible by injury, *e.g.*, ischemic injury, or ischemia. As used herein, "ischemia" means having a blood flow at least 10% below that which is normal for an individual of similar size and age, as measured under resting conditions or exercise conditions. In an adult human, normal resting blood flow is approximately 1 ml/min/gram of myocardial

mass. During exercise, blood flow typically rises to approximately 3-6 ml/min/gram of myocardial mass. Ischemia may be associated with a physical wound or blow, sudden loss of blood volume, toxicity, or a physical obstruction such as a tumor.

Other types of injury include, *e.g.*, injury due to hypertension, chemotherapy (*e.g.*, injury due to cisplatin damage), chronic renal failure, injury due to auto-immune disorders (*e.g.*, lupus), or polycystic kidney (PCK) disease.

In some embodiments, the regulatory sequence preferentially directs expression of an operably linked sequence in renal tissue. The term "operably linked" means that the regulatory sequence is associated with the nucleic acid in such a manner as to facilitate transcription of the nucleic acid. In some embodiments, the operably linked nucleic acid encodes an antisense nucleic acid. The antisense nucleic acid can be a portion of the anti-sense strand of a gene whose expression is intended to be decreased in a renal tissue. For example, for conditions characterized by undesired proliferation of kidney tissue, the DNA may be a KIM-1 antisense nucleic acid.

In other embodiments, the DNA is operably linked to at least one polypeptide-encoding sequence. The polypeptide sequence can be, *e.g.*, one encoded by a KIM-1 cDNA. Examples of nucleic acids encoding rat and human KIM-1 cDNAs, and their corresponding encoded amino acid sequences are provided in PCT publication WO97/44460.

Alternatively, the DNA is operably linked to nucleic acid that encodes a polypeptide other than KIM-1. For example, the polypeptide can be a therapeutic factor such as insulin growth factor (IGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF β) Type II receptor, particularly the soluble fragment of TGF β receptor, hepatocyte growth factor (HGF), and the endothelial cell adhesion molecule ICAM-1. Other therapeutic polypeptides include factors such as a cell survival-promoting factor, a cell growth-promoting factor, a wound-healing factor, an anti-fibrotic factor, an apoptosis-inhibiting factor, an anti-inflammatory factor, a terminal differentiation-promoting factor, a cell growth-inhibiting factor, an intravascular-volume restoration factor, a chelating agent, an alkylating agent, an angiotensin-converting enzyme-inhibiting factor, erythropoietin, a cytokine, a receptor, an anticoagulant, an enzyme, a hormone, an antibody, and a renal structural protein.

A nucleic acid to be transcribed from a KIM-1 derived regulatory element can also be linked to a reporter gene. A reporter gene is any gene encoding a protein, the amount of which can be determined. Exemplary reporter genes include the luciferase gene, *e.g.*, the bacterial

luciferase gene, *e.g.*, the luciferase gene present in pGL3-basic (Promega Corp., Madison, WI). Other suitable reporter genes include the beta-galactosidase gene (LacZ), the chloramphenicol acetyl transferase (CAT) gene, or any gene encoding a protein providing resistance to a specific drug.

5 The regulatory elements disclosed herein can also be used to prepare probes and primers based on KIM-1 derived regulatory sequences. These probes and primers can be used, *e.g.*, to identify KIM-1 genomic regions in a subject, such as a human. The probes can be provided in the form of a probe or primer that includes a region of nucleotide sequence that hybridizes under stringent conditions to at least approximately 6, 8, 10 or 12, preferably about
10 25, 30, 40, 50 or 75 consecutive nucleotides of any of SEQ ID NOS: 1, 2, 3, or 4.

The probe optionally includes an attached label, which is capable of being detected. The label can be, *e.g.*, radioisotopes, fluorescent moieties, enzymes, and enzyme co-factors.

The *cis*-acting regulatory sequences, including the probe or primer molecules, can also be used as a part of a diagnostic test kit, for example, to detect mutations in the promoter,
15 which result in faulty expression of a renal gene or a gene associated with renal tissue.

Nucleic acids, including nucleic acid fragments, containing or derived from *cis*-acting KIM-1 regulatory sequences can be prepared according to methods well known in the art and described, *e.g.*, in Sambrook, J. Fritsch, E. F., and Maniatis, T. (1989) MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20 For example, discrete fragments of the regulatory element can be prepared and cloned using restriction enzymes. Alternatively, discrete fragments can be prepared using the Polymerase Chain Reaction (PCR) using primers having an appropriate sequence, such as a sequence in SEQ ID NO: 1. The activity of promoter fragments can be tested in vitro in transfection assays or in vivo in transgenic animals described herein. Also within the scope of the invention are
25 nucleic acids that are homologues or equivalents of the above-described nucleic acids.

Cis-acting KIM-1 derived regulatory sequences can be isolated from other organisms by using a KIM-1 cDNA to screen genomic DNA sequences in the organism of interest, and then testing the genomic sequences in promoter-reporter assays as described herein. Preferably, the KIM-1 cDNA used for the screening is from the same, or closely related
30 organism. Thus, to isolate a murine KIM-1 derived *cis*-regulatory sequence, the murine KIM-1 cDNA is used. Preferably, the probe is derived from a 5' region of the KIM-1 cDNA.

Vectors and cells containing *cis*-acting KIM-1 derived regulatory sequences

This invention also provides vectors, *e.g.*, expression vectors that include *cis*-acting KIM-1 derived regulatory sequences.

In some embodiments, the expression vector includes a recombinant gene encoding a KIM-1 or a therapeutic polypeptide. Such expression vectors can be used to transfect cells and thereby produce protein. Constructs containing *cis*-acting KIM-1 derived regulatory sequences can also be used as a part of a gene therapy protocol to deliver nucleic acids *in vitro* or *in vivo* to particular cell types (*e.g.*, kidney).

The vector can include any vector known in the art for propagating a desired nucleic acid in a cell of interest. Thus, the vector can be chosen to propagate a nucleic acid that includes a *cis*-acting KIM-1 derived regulatory sequences in a prokaryotic or eukaryotic host, or both. In some embodiments, the vector is a viral vector, *e.g.*, a retroviral vector. For a review, see Miller, A. D. (1990) *Blood* 76:271. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found, *e.g.*, in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, F. M. *et al.* (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM.

Vectors can alternatively be adenovirus-derived vectors, *e.g.*, those described in Berkner *et al.* (1988) *BioTechniques* 6:616; Rosenfeld *et al.* (1991) *Science* 252:431-434; and Rosenfeld *et al.* (1992) *Cell* 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art.

The vector can be derived from an adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. For a review see Muzyczka *et al.* *Curr. Topics in Micro. and Immunol.* (1992) 158:97-129. It is also one of the few viruses that can integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte *et al.* (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski *et al.* (1989) *J. Virol.* 63:3822-3828; and McLaughlin *et al.* (1989) *J. Virol.* 62:1963-1973). See also U.S. Patent No. 5,872,154. Other suitable vectors include those based in the human immunodeficiency virus (HIV). These vectors are described in, *e.g.*, U.S. Patent No. 5,665,577 and U.S. Patent No. 5,981,276.

Vectors can be introduced into cells using methods known in the art. In addition to viral transfer methods, such as those illustrated above, non-viral methods can also be used to introduce a gene. These methods include, *e.g.*, calcium phosphate precipitation, microparticle-mediated delivery, and biolistic transformation. In some embodiments, delivery can rely on endocytic pathways for the uptake of genes by the targeted cell. Exemplary targeting means of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

Delivery can be performed using nucleic acids entrapped in liposomes bearing positive charges on their surface (*e.g.*, lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target tissue. See, *e.g.*, Mizuno *et al.* (1992) *No Shinkei Geka* 20:547-551; PCT publication WO91/06309; Japanese patent application 1047381; and European patent publication EP-A-43075. For example, lipofection of cells can be carried out using liposomes tagged with monoclonal antibodies against any cell surface antigen present on a hepatic cell, such as an asialoglycoprotein receptor.

Cells containing *cis*-acting KIM-1 regulatory sequences, or vectors that include *cis*-acting KIM-1 regulatory sequences as described herein, can be any cell known in the art. Thus, they can include prokaryotic cells (*e.g.*, *E. coli* cells) or eukaryotic cells. Eukaryotic cells can include single-celled organisms such, *e.g.*, yeast (*e.g.*, *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*). Alternatively, the cells can be mammalian cells, *e.g.*, human or simian cells. In some embodiments, the cells are kidney cells, or cell lines derived from kidney cells.

Transgenic animals containing *cis*-acting KIM-1 regulatory sequences

The invention also includes transgenic non-human vertebrates, *e.g.*, mammals and birds, that contain *cis*-acting KIM-1 regulatory sequences.

For example, some embodiments, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which exogenous *cis*-acting KIM-1 regulatory sequences have been introduced. Such host cells can be used to create non-human transgenic vertebrate animals in which exogenous *cis*-acting KIM-1 regulatory sequences have been introduced into their genome or homologous recombinant animals in which endogenous *cis*-acting KIM-1 regulatory sequences have been altered. Such animals are useful for studying the function and/or activity of *cis*-acting KIM-1 regulatory sequences and for identifying and/or evaluating modulators of *cis*-acting KIM-1 regulatory sequences. As used herein, a "transgenic animal"

means a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, zebrafish, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse or a rat, in which an endogenous *cis*-acting KIM-1 regulatory sequences has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing *cis*-acting KIM-1 regulatory sequences into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, or the like, and allowing the oocyte to develop in a pseudopregnant female foster animal. For example, a *cis*-acting KIM-1 regulatory sequence having the nucleic acid sequence of SEQ ID NO:3, or a functional fragment thereof, can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human *cis*-acting KIM-1 regulatory sequences, such as a *cis*-acting KIM-1 regulatory sequence, can be isolated based on hybridization to the human *cis*-acting KIM-1 regulatory sequences (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1996). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the transgenic *cis*-acting KIM-1 derived regulatory sequences in its genome and/or expression of sequences operably linked to the transgenic *cis*-acting KIM-1 derived regulatory sequences. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene carrying a transgenic *cis*-acting KIM-1 derived regulatory sequence can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a *cis*-acting KIM-1 regulatory sequences gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the *cis*-acting KIM-1 regulatory sequences. The *cis*-acting KIM-1 regulatory sequence can be a human sequence (*e.g.*, SEQ ID NO:3), but more preferably, is a non-human homologue of a human *cis*-acting KIM-1 regulatory sequence. For example, a mouse homologue of human *cis*-acting KIM-1 regulatory sequence of SEQ ID NO:3 can be used to construct a homologous recombination vector suitable for altering a *cis*-acting KIM-1 regulatory sequence in the mouse genome.

10 In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous a *cis*-acting KIM-1 regulatory sequence is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous *cis*-acting KIM-1 regulatory sequence is mutated or otherwise altered but is still functional (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous *cis*-acting KIM-1 regulatory sequence). In the homologous recombination vector, the altered portion of the *cis*-acting KIM-1 regulatory sequence is flanked at its 5' and 3' ends by additional nucleic acid of the *cis*-acting KIM-1 regulatory sequence to allow for homologous recombination to occur between the exogenous *cis*-acting KIM-1 regulatory sequence carried by the vector and an endogenous *cis*-acting KIM-1 regulatory sequence in an embryonic stem cell. The additional flanking *cis*-acting KIM-1 regulatory sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced *cis*-acting KIM-1 regulatory sequence has homologously recombined with the endogenous *cis*-acting KIM-1 regulatory sequence are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ

cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, e.g., Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyst and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

Identifying trans-acting factors that bind to cis-acting KIM-1-derived regulatory sequences

Also provided are methods of identifying compounds that bind to cis-acting KIM-1-derived regulatory sequences. These compounds include trans-acting factors can include, e.g., polypeptides such as transcription factors, which interact preferentially with cis-acting KIM-1 regulatory sequences, or small molecules.

In one embodiment, a compound is identified by performing assays in which a *cis*-acting KIM-1 nucleic acid sequence is incubated with a test compound. Binding of the compound to the nucleic acid is detected using methods known in the art for assessing nucleic acid binding. For example, binding can be measured using electrophoretic mobility shift assays (EMSA). One way in which an EMSA can be prepared is to incubate together a DNA, which is preferably labeled, containing a KIM-1-derived *cis*-acting regulatory sequence and the test compound. The mixture is then subjected to electrophoresis, and the migration of the labeled nucleic acid in the presence of the test compound is compared to the migration of the labeled nucleic acid in the absence of the test compound. A difference in mobility indicates that the test compound binds to regulatory sequence.

Any suitable compound can be used as the test compound. In some embodiments, the test compound is obtained from a cellular extract known to contain, or to be suspected of containing, a *trans*-acting factor. Suitable cells include kidney cells, *e.g.*, Cos cells.

Cell-based methods can also be used to identify compounds that modulate activity. For example, a cell containing a *cis*-acting KIM-1-derived regulatory sequence operably linked to a nucleic acid encoding a reporter molecule is contacted with a test compound and the reporter molecule mRNA or translated product is measured. mRNA levels and protein levels can be determined using any method known in the art, *e.g.* using Northern blot hybridization analysis, immunoprecipitations, or immunohistochemistry.

The *trans*-acting factors can also be identified using *in vivo* assays. For example, a reporter construct can be constructed in which a reporter gene is under the control of any of the *cis*-acting KIM-1-derived regulatory sequences disclosed herein.

The reporter gene can be any gene encoding a suitably detectable protein. The reporter gene can be, *e.g.*, a gene encoding luciferase. Cells are transfected with the reporter construct that includes a *cis*-acting KIM-1 regulatory element. Transfection can be transient or stable. The cells can be transfected with more than one reporter construct. The transfected cells can then be incubated in the presence or absence of a test compound for an appropriate amount of time and the level of expression of the reporter gene is determined.

Similar assays can also be performed using a cell or nuclear extract instead of cells. Thus, in one embodiment, the invention provides a method for identifying a compound which modulates KIM activity. The method includes incubating a reporter construct that includes any of the regulatory elements according to the invention with a nuclear or cellular extract, or isolated nuclei, in the presence or absence of test compound. Expression of the test compound

is then measured, *e.g.*, by including a labeled nucleotide in the reaction and measuring the amount of label incorporated in the product transcribed from the reporter construct. Other methods can also be used to determine the amount of reporter gene expression in this system, such as the measure of the amount of protein expressed by the reporter gene.

5 In yet another embodiment, compounds that modulate the regulatory elements of the present invention *in vivo* can be identified in non-human animals. In one embodiment of the invention, a non-human animal, *e.g.*, a mouse, is treated with a compound, such as a compound identified in one of the assays described above. After an appropriate amount of time, the level of activity is determined and compared to its activity in a mouse that has not received the test
10 compound.

Pharmaceutical compositions containing *cis*-acting KIM-1 regulatory sequences

Pharmaceutical compositions containing *cis*-acting KIM-1 regulatory sequences can be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients.

15 Administration can be parenteral, intravenous, subcutaneous, intramuscular, retroperitoneal, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, transmucosal, or oral. The Nucleic acids can be provided in compositions formulated in various ways, according to the corresponding route of administration. For example, liquid solutions can be made for ingestion or injection. Gels or
20 powders can be made for ingestion, inhalation, or topical application. Methods for making such formulations are well known and can be found in standard references in the field, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, Mack Publishing Company, Easter, Pa., 15th Edition (1975).

25 The compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

30 The compositions can be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example

comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The therapeutic compositions can also contain a carrier or excipient. Useful excipients include buffers (for example, citrate buffer, phosphate buffer, acetate buffer, and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (for example, serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol.

Methods for using *cis*-acting KIM-1 regulatory sequences

Cells containing *cis*-acting KIM-1 regulatory sequences operably linked to a polypeptide-encoding sequence can be used to direct expression of the polypeptide. For example, the polypeptide can be expressed by providing a cell containing a KIM-1 regulatory sequence and culturing the cell, if necessary, to allow for expression of the polypeptide. Any cell type can be used as long as it allows for expression of the polypeptide-encoding sequence operably linked to the *cis*-acting regulatory sequence. In preferred embodiments, the cell is a renal cell.

In some embodiments, the expressed polypeptide is isolated. If desired, the polypeptide-encoding sequence can include a sequence encoding a signal sequence to allow for secretion of the polypeptide. The polypeptide can then be isolated from the extracellular medium.

The *cis*-acting KIM-1 regulatory sequences can also be used to increase transcription of an operably linked sequence *in vitro* or *in vivo*, *e.g.*, in a cell, tissue or subject (such as a human). The operably linked sequence can be, *e.g.*, a polypeptide-encoding sequence or an antisense nucleic acid construct. To increase transcription, a cell containing a *cis*-acting KIM-1 regulatory sequence operably linked to the sequence of interest, or a tissue containing two or more of such cells, is cultured under conditions that allow for the expression of the operably linked sequence to allow for increased levels of transcripts corresponding to the operably linked sequence in the cell or tissue. "Culture" as used herein can include *in vitro* culture under conditions necessary for maintaining the viability of mammalian cells, or *in situ* culture of cells in the body of an animal.

In another embodiment, the *cis*-acting KIM-1 regulatory sequences are used to direct expression of a nucleic acid sequence that is not normally under the control of such regulatory sequences. A nucleic acid molecule containing a *cis*-acting KIM-1 regulatory sequence is integrated into the genome of a target cell in the vicinity of a gene of interest. The gene of

interest is preferably one that is normally not expressed in renal tissue, or is expressed at low amounts in renal tissue. Integration of the introduced *cis*-acting KIM-1 regulatory sequence near the gene of interest allows for the expression of the gene of interest under the control of the KIM-1 regulatory sequence. Preferably, the *cis*-acting KIM-1 regulatory sequences are
5 introduced near the 5' region of the gene of interest.

Another use of the *cis*-acting KIM-1 regulatory sequences is to deliver a therapeutic polypeptide to renal tissue of a subject. To deliver the polypeptide, a cell including a *cis*-acting KIM-1 regulatory sequence operably linked to a therapeutic polypeptide-nucleic acid sequence is introduced into renal tissue. The sequences are expressed, *e.g.*, by culturing the cell under
10 conditions that allow for the expression of the linked polypeptide, to result in the delivery of the therapeutic polypeptide to the subject's renal tissue. In some embodiments, the therapeutic polypeptide linked to the *cis*-acting KIM-1 regulatory sequence is expressed following a stimulus. The stimulus can be, *e.g.*, an injury such as ischemic injury or is ischemic reperfusion injury, or some other nephrotoxic injury.

The *cis*-acting KIM-1 regulatory sequences can also be used in a method for treating or preventing renal tissue injury in a subject. The method can include providing a cell in the subject that includes an introduced *cis*-acting KIM-1 regulatory sequence operably linked to a nucleic acid encoding a therapeutic nucleic acid, *e.g.*, a therapeutic polypeptide-encoding sequence. The nucleic acid is allowed to express, and the gene product thereby introduced to
20 the renal tissue to prevent or treat renal tissue injury in the subject. The *cis*-acting KIM-1 regulatory sequence can be introduced into the subject using methods described in the art for introducing nucleic acid sequences into cells. The nucleic acids can be introduced *ex vivo* or *in vivo*.

For gene therapy or antisense therapy, the claimed DNA may be introduced into target
25 cells of an animal, *e.g.*, a patient, using standard vectors and/or gene delivery systems. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses, and adeno-associated viruses, among others. Delivery of nucleic acids to a specific site in the body for gene therapy or antisense therapy may also be accomplished using a biolistic delivery system,
30 such as that described by Williams *et al.*, 1991, *Proc. Natl. Acad. Sci. U.S.A.* 88:2726-2729. Standard methods for transfecting cells with isolated DNA are well known to those skilled in the art of molecular biology. Gene therapy and antisense therapy to prevent or decrease the development kidney disease or injury may be carried out by directly administering the claimed

DNA to a patient or by transfecting renal cells with the claimed DNA ex vivo and infusing the transfected cells into the patient.

A therapeutically effective amount is an amount of the DNA of the invention that is capable of producing a medically desirable result in a treated animal. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{22} copies of the DNA molecule.

Nucleic acids can be delivered to a subject by any of a number of routes, *e.g.*, as described in U.S. Patent Nos. 5,399,346 and 5,580,859. Delivery can thus also include, *e.g.*, intravenous injection, local administration, and systemic administration (see U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057).

The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can include a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

EXAMPLES

The following examples illustrate particular non-limiting embodiments of the invention.

Example 1 - Cloning and Characterization of KIM-1 derived sequences

Cis-acting KIM-1 derived regulatory sequences were identified by screening a human genomic library with a 220 bp NotI-KpnI DNA fragment containing sequences in the 5' region of the human KIM-1 cDNA. The human KIM-1 cDNA is described generally in PCT publication WO97/44460 and Ichimura *et al.*, *J. Biol. Chem.* 273:4135-42, 1998.

The screening identified a genomic fragment of 8933 bp (SEQ ID NO:1). The sequence of the 8933 bp region is shown in FIGS. 1A-1H. A translational start codon is present beginning at nucleotide 8655.

A 4817 bp KpnI-KpnI fragment (SEQ ID NO:2), which corresponds to nucleotides 3796-8612 of FIGS. 1A-1H, was subcloned into a luciferase-encoding pGL3b expression

vector (Promega Corp.) and named p4.8KIM/pGL3b. The p4.8KIM/pGL3K construct is shown schematically in FIG. 2B. The presence of the 4817 bp KpnI-KpnI fragment in the pLUC3 was found to increase levels of encoded luciferase in renal cells, as is explained in more detail in the Examples, below.

5 A smaller sequence able to increase expression of luciferase in kidney cells was identified by subcloning a 1289 bp EcoRI-KpnI fragment (SEQ ID NO:3) corresponding to nucleotides 7322-8612 in FIG. 1 into a pLUC3 vector. The resulting construct was named 1.3pKIM-1, and is shown schematically in FIG. 2C.

A construct named 0.5pKIM, a pLUC3-based construct including only nucleotides 10 8110 to 8612 (SEQ ID NO:4) of FIG. 1, did not increase levels of luciferase as compared to expression. However, even though the region 8110 to 8612 is inactive alone, sequences present within the region may nevertheless be required, along with other sequences, to confer renal cell expression of linked sequences.

15 **Example 2 - Expression in kidney cells of reporter sequences operably linked to KIM-1 derived sequences**

Human genomic sequences from the 5' region of the human KIM-1 gene were tested for their ability to direct expression of a reporter polypeptide in three kidney-derived cell lines COS cells, a cell line derived from African green monkey kidney fibroblasts; LC/PK cells, a cell line derived porcine kidney epithelial cell lines; and MDCK cells, a cell line derived from 20 canine kidney epithelial cells.

The cell lines were transiently transfected with constructs containing various regions of DNA from the human KIM-1 gene linked to a reporter luciferase gene. These constructs were concomitantly transfected with pCMV driven β -galactosidase vectors to standardize transfection efficiency, and activity of luciferase and β -galactosidase was measured. Activities 25 were calculated as luciferase/ β -gal ratios. Relative activities were calculated as the ratio of construct activity to negative control, *i.e.*, promoterless luciferase vector (pGL3b).

DEAE-mediated transfection was used to introduce constructs (described in more detail below) into cell lines. Transfection was performed at 80% confluence, about 24 hours after seeding cells.

30 DNA was introduced into the cells by aspirating medium from the cells and mixing 10 ml of the appropriate culture medium (including 10% Nu serum, Collaborative Biomedical Products, #51004), 400 μ l of DEAE (1X PBS + 10 mg/ml DEAE Dextran + 2.5 mM

Chloroquine), and 20 µg of DNA (10 µg luciferase construct, 2 µg /β-gal vector, 8 µg BlueScript Vector).

Cells were exposed to DNA for 2-4 hours, after which the DNA solution was removed by aspiration and replaced with 5 ml 10% DMSO in 1X PBS. After 2 minutes, this solution
5 was removed and the cells were washed twice with 1X PBS. Fresh medium was added, and cells were harvested after 48 hours.

Cells were washed once with 1X PBS, then incubated with 500 µl of 1X PBS. Cells were collected and centrifuged for 2 minutes, after which the supernatant was removed. Cells were resuspended in 200 µl of 0.25M TRIS, pH 7.8, and subjected to 3 shock freeze-thaw
10 cycles, then centrifuged for 5 minutes at 14,000 g. The supernatant was used for luciferase and β Galactosidase assays.

To measure β-galactosidase activity, 25 µl of supernatant, 30 µl of 10X Mg buffer (90 mM MgCl₂, 1.02M beta-mercaptoethanol), 60 µl of 40 mM CPRG, and 48 µl of 0.5 sodium phosphate pH 7.5 were mixed and incubated until a red color developed. 500 µl of 1M
15 Na₂CO₃ was added, and the OD at 570 nm was measured.

To measure luciferase activity 25 µl (p10) of the supernatant were mixed with 50 µl of 2X assay buffer, as per the manufacturer's instructions (Catalog # E 1502, Promega Corporation, Madison, WI). Measurements were made using a photoluminometer.

The KIM-1 human genomic region used to generate the constructs is shown in FIG. 2A.
20 Constructs used in the transfection assays are shown in FIGS. 2B-2D. An 8933 bp fragment (SEQ ID NO:1) is shown schematically in FIG. 2A in a 5' to 3' orientation. KpnI sites are located at positions 3796 and 8612 as shown in the figure. For reference, the ATG initiation codon of the human KIM-1 occurs at position 8655.

The tested sequences are shown schematically in FIGS. 2B-2D. One tested sequence
25 included a 4816 bp KpnI-KpnI fragment from the 5' flanking region of the human KIM-1 gene. This fragment corresponds to the sequences bordered by KpnI sites at positions 3796 and 8612 of MZ007. FIG. 2B illustrates a construct made by inserting the 4816 bp KpnI-KpnI fragment into a pLUC3 Basic expression vector (Promega Corporation). The resulting construct was named 4.8 pKIM/PGL3b.

30 A shorter fragment from the 4816 bp KpnI-KpnI region was also tested. This fragment was defined by an EcoRI-KpnI fragment encompassing nucleotides 7322 -8611 of MZ007.

This KpnI fragment was inserted into the pLUC3 Basic expression vector and named 1.3pKIM/pGL3b. This construct is shown schematically in FIG. 2C.

A still shorter fragment defined by a SacII-KpnI fragment encompassing nucleotides 8110-8612 of MZ007 was also examined. This construct was named 0.5pKIM/pGL3b and is shown schematically in FIG. 2D.

For each cell line tested, relative luciferase activities were calculated by measuring luciferase following transfection with no plasmid (*i.e.*, zero, "0"), the pGL3 Basic vector alone ("pGL3"), or the constructs 4.8pKIM/pGL3b, 1.3pKIM/pGL3b, and 0.5pKIM/pGL3b.

For transformations into COS cells, DMEM was used as the culture medium. For the 4.8pKIM construct, four trials were performed, each using 2 plates. The RA was 3.8, with SD of 2.82. For the 1.3 pKIM construct, four trials were performed, each using 2 plates. The RA was 6.3, with an SD of 4.19. For the 0.5 pKIM construct, 2 trials were performed, each using 2 plates. The RA as 3.3, with an SD of 1.54. The results of the transfection assays using the COS cells are shown in FIG. 3.

For transformations into LLC-PK cells, DMEM was used as the culture medium. For the 4.8pKIM/PGL3b construct, three trials were performed, each using 2 plates. The RA was 5.5, with a SD of 1.43. For the 1.3pKIM/PGL3b construct, three trials were performed, each using 2 plates. The RA was 3.4, with an SD of 0.89. For the 0.5pKIM/PGL3b construct, two trials were performed, using 2 plates each. The RA was 1.5, with an SD of 0.34. The results of the transfection assays using LLC-PK cells are summarized in FIG. 4.

For transformations into MDCK cells, MEM was used as the culture medium. For the 4.8pKIM construct, 3 trials were performed, using 2 plates each. The relative activity (RA) was 7.0, and the SD was 3.46. For the 1.3 pKIM/PGL3b construct, 3 trials were performed, each using 2 plates. The relative activity was 3.4, with a SD of 0.89. The results for the transfection assays using MDCK cells are shown in FIG. 5.

In assays using COS cells (FIG. 3), LLC/PK1 cells (FIG. 4), and MDCK cells (FIG. 5), luciferase activity was significantly higher in cells transfected with the constructs 4.8pKIM/pGL3b and 1.3pKIM/pGL3b as compared to cells transfected with pGL3 alone, and cells not transfected with any construct.

These results indicate that sequences nucleotides 7322-8612 as shown in FIGS. 1A-H contain *cis*-acting regulatory elements that are able to increase expression of operably linked sequences in kidney tissues. Additional elements that increase this activity may be in 3796-7322 region, as is shown in FIG. 6, as is discussed in Example 3, below.

The 0.5pKIM/pGL3b construct did not increase expression of luciferase relative to cells transfected with pGL3 alone in the cell types tested. While these results suggest that these KIM-1 derived sequences did not confer expression of linked sequences, at least in the cell lines and conditions used, these results do not exclude the possibility that this region of the KIM-1 flanking region contains elements that are necessary or important for increasing expression of linked sequences in kidney tissues.

Example 3 - Expression in kidney cells of reporter sequences operably linked to KIM-1 derived sequences following injury

Expression of luciferase encoded by 4.8pKIM, 1.3 pKIM, and 0.5 pKIM was measured in transfected HK2 cells that had been subjected to chemical anoxia using cyanide and deoxyglucose. HK2 cells are derived from epithelial proximal tubule cells from human kidney.

A 12-well plate system (MULTIWELL™ 12-well, Becton-Dickson) was used in these studies. The culture medium used was EGM (Clontech). Prior to transformation, 2 ml medium per well was added to the cells, then removed by aspiration. HK2 cells were seeded at a density of 30,000/ well. Cells reached 80% confluence after 16-24 hours.

DNA was prepared by mixing 50 µl of serum free medium, 5 µg of DNA (2.5 µg luciferase construct, 0.5 µg β-galactosidase vector, 2 µg BlueScript vector), and 5 µl of SUPERFECT reagent (Qiagen Corp.), and incubating for 7 minutes. 300 µl of medium + 10% FCS was added, and the mix was then added to the cells. The cells were then allowed to incubate for 2 hours. The DNA solution was next removed by aspiration, after which the cells were washed twice in 1X PBS, after which the cells were incubated in culture medium with 10% FCS.

To induce chemical anoxia, medium was removed by aspiration, and cells were washed once with 1X PBS. Cells were then incubated for 90 minutes in 1 ml Krebs-Henseleit buffer ("KHB") (6.72 mM NaCl, 3.6 mM KCl, 1.3 mM KH₂PO₄, 25 mM NaHCO₃, 1 mM CaCl₂, a mM MgCl₂, pH 7.4 in incubator), along with 5 mM sodium cyanide and 5 mM deoxyglucose. Cells were then washed once with 1X PBS and incubated in KHB + 10mM dextrose for 15-20 minutes, then harvested as explained below.

For harvesting, cells were washed once in 1X PBS, then incubated for 5 minutes with 200 µl 25 mM GlyGly, 15 mM MgSO₄, 4 mM EGTA, pH 8.0, 1% Triton X 100, 1 mM DTT. Lysate was then removed from the wells and used for luciferase and β galactosidase assays.

To assay for β galactosidase activity, 50 μ l of cell extract was mixed with 50 μ l of assay buffer (Catalog # E2000, Promega Corporation, Madison, WI), followed by incubation at 37° C until a faint yellow color developed. Signals were then measured in an ELISA reader at 405 nm.

5 Luciferase activity was measured by mixing 50 μ l of 2X assay buffer as indicated by the manufacturer (Catalog #E1502, Promega, Madison, WI). Measurements were made using a photoluminometer.

Luciferase expression was measured in three different populations of transfected HK2 cells. The first group included cells not subjected to chemical anoxia. These cells were
10 assayed 72 hours following transfection. The second group included cells assayed 72 hours following transfection and 90 minutes after inducement of chemical anoxia. For each group of cells, separate populations of cells were transfected with 4.8pKIM/pGL3b, 1.3pKIM/GL3b, and 0.5pKIM/pGL3b.

The results are illustrated in FIG. 6. For baseline cells, the 4.8pKIM/pGL3b construct
15 yielded a RA of 9.1, with a SEM of 1.1. The 1.3pKIM/pGL3b construct yielded a RA of 5.0, with a SEM of 0.8. The 0.5 pKIM constructed yielded a RA of 1.6, with a SEM of 0.2.

In cells subjected to chemical anoxia, the 4.8pKIM/pGL3b generated a RA of 13.5, with a SEM of 1.5. The 1.3pKIM/pGL3b construct yielded a RA of 5.6, with a SEM of 0.4. The 0.5pKIM/pGL3b construct generated a RA of 2.0, with a SEM of 0.3.

20 These data demonstrate that KIM-1 sequences present in the 4.8pKIM/pGL3b construct caused higher levels of expression of the linked luciferase gene in cells subjected to anoxia, as compared to control cells. This effect was not seen with the 1.3pKIM/pGL3b and 0.5pKIM/pGL3b constructs in this experiment.

25 **Example 4 -Expression in confluent kidney cells of reporter sequences operably linked to KIM-1 derived sequences**

Expression of a luciferase gene linked to 4.8pKIM/PGL3b, 1.3pKIM/PGL3b, or 0.5pKIM/PGL3b in confluent cells was investigated.

MDCK cells were transfected with the indicated construct 16-24 hours after seeding. Transfected cells were harvested after 24, 48, and 72 hours, and luciferase and β -galactosidase
30 activity was then measured. The results are summarized below:

Table 1

	RA	SD
24 hours, 80% confluence		
4.8pKIM/PGL3b	5.65	0.97
1.3pKIM/PGL3b	4.93	2.07
0.5pKIM/PGL3b	2.03	.98
48 hours, 90% confluence		
4.8pKIM/PGL3b	13.55	3.5
1.3pKIM/PGL3b	7.95	1.91
0.5pKIM/PGL3b	2.4	.61
72 hours, 100% confluence		
4.8pKIM/PGL3b	31.8	6.5
1.3pKIM/PGL3b	9.83	4.82
0.5pKIM/PGL3b	1.93	1.09

These results demonstrated that KIM-1 sequences present in 4.8pKIM/pGL3B lead to significantly higher levels of luciferase as cells reach confluency. KIM-1 sequences present only in the 1.3pKIM/pGL3B and 0.5pKIM/pGL3B constructs did not increase expression of luciferase in these studies.

Example 5 - Use of KIM-1-derived *cis*-acting regulatory sequences in gene therapy

KIM-1 derived *cis*-acting regulatory sequences according to the invention can be used for gene therapy treatment of renal diseases. KIM-1 *cis*-acting regulatory sequences can be used alone or as part of a vector to express heterologous genes, *e.g.*, a KIM-1 cDNA, or a protein other than a KIM-1 polypeptide, in renal cells.

The DNA or vector containing a KIM-1 *cis*-acting regulatory sequence linked to a nucleic acid encoding a polypeptide of interest is introduced into renal cells, which in turn produce the polypeptide of interest. For example, sequences encoding the desired polypeptide may be operably linked to the renal cell-specific promoter sequences of the invention and expressed in renal cells.

Example 6 - Use of KIM-1-derived *cis*-acting regulatory sequences in antisense therapy

The KIM-1 *cis*-acting regulatory sequence is used in methods of antisense therapy. Antisense therapy is carried out by administering to an animal, *e.g.*, a human patient, DNA containing the renal cell-specific promoter sequences of the invention operably linked to a DNA sequence, *i.e.*, an antisense template, which is transcribed into an antisense RNA. The

antisense RNA is a short nucleotide sequence (generally at least 10 nucleotides, preferably at least 14 nucleotides, and up to 100 or more nucleotides) formulated to be complementary to a portion of a specific mRNA sequence. The antisense template is preferably located downstream from the promoter sequences of the invention. A poly A tail element is typically
5 located at the end of the antisense sequence to signal the end of the sequence. Standard methods relating to antisense technology have been described. See, *e.g.*, Melani *et al.*, *Cancer Res.* 51:2897-2901, 1991. Following transcription of the DNA sequence into antisense RNA, the antisense RNA binds to its target mRNA molecules within a cell, thereby inhibiting translation of the mRNA and down-regulating expression of the protein encoded by the
10 mRNA.

The expression of other renal cell proteins may also be inhibited in a similar manner. For example, the DNA of the invention can be operably linked to antisense templates that are transcribed into antisense RNA capable of inhibiting the expression of the following proteins: TGF- β , dysfunctional collagen mutant genes, WT-1 (Wilms Tumor gene), and genes
15 associated with polycystic kidney disease (PCK).

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages,
20 and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated DNA comprising a *cis*-acting KIM-1 derived regulatory sequence.
2. The DNA of claim 1, wherein said DNA comprises SEQ ID NO:3.
3. The DNA of claim 1, wherein said DNA comprises SEQ ID NO:2.
4. The DNA of claim 1, wherein said regulatory sequence preferentially directs expression of an operably linked sequence in renal tissue.
5. The DNA of claim 1, wherein said regulatory sequence is inducible.
6. The DNA of claim 5, wherein said regulatory sequence is inducible by injury.
7. The DNA of claim 6, wherein said injury is ischemic.
8. The DNA of claim 1, wherein said DNA comprises at least 5 contiguous nucleotides from SEQ ID NO:3, or sequences complementary to SEQ ID NO:3.
9. The DNA of claim 1, wherein said DNA comprises at least 5 contiguous nucleotides from a sequence that hybridizes with SEQ ID NO:3, or sequences complementary to SEQ ID NO:3.
10. The DNA of claim 8, wherein said DNA comprises between 5 and 35 contiguous nucleotides from SEQ ID NO:3, or sequences complementary to SEQ ID NO:3.
11. The DNA of claim 9, wherein said DNA comprises between 5 and 35 contiguous nucleotides from a sequence that hybridizes with SEQ ID NO:3, or sequences complementary to SEQ ID NO:3.
12. The DNA of claim 1, wherein said DNA is operably linked to a sequence encoding a KIM-1 antisense nucleic acid.

13. The DNA of claim 1, wherein the DNA is operably linked to at least one polypeptide-encoding sequence and regulates renal tissue-specific transcription of said polypeptide-encoding sequence
14. The DNA of claim 13, wherein said DNA comprises a portion of SEQ ID NO:3 that is sufficient to regulate kidney tissue-specific transcription of said polypeptide-encoding sequence.
15. The DNA of claim 13, wherein said regulatory sequence is inducible.
16. The DNA of claim 13, wherein said polypeptide-encoding sequence encodes a KIM-1 polypeptide.
17. The DNA of claim 16, wherein said KIM-1 polypeptide comprises the amino acid sequence of a human KIM-1 polypeptide.
18. The DNA of claim 13, wherein said polypeptide-encoding sequence does not encode a KIM-1 polypeptide.
19. The DNA of claim 13, wherein said polypeptide-encoding sequence encodes a therapeutic polypeptide.
20. The DNA of claim 13, wherein said polypeptide is selected from the group consisting of a cell survival-promoting factor, a cell growth-promoting factor, a wound-healing factor, an anti-fibrotic factor, an apoptosis-inhibiting factor, an anti-inflammatory factor, a terminal differentiation-promoting factor, a cell growth-inhibiting factor, an intravascular-volume restoration factor, a chelating agent, an alkylating agent, an angiotensin-converting enzyme-inhibiting factor, erythropoietin, a cytokine, a receptor, an anticoagulant, an enzyme, a hormone, an antibody, and a renal structural protein.

21. The DNA of claim 13, wherein said polypeptide is selected from the group consisting of an insulin growth factor (IGF), an epidermal growth factor (EGF), a fibroblast growth factor (FGF), a transforming growth factor beta (TGF β) Type II receptor, a hepatocyte growth factor (HGF), and an endothelial cell adhesion molecule ICAM-1.
22. A vector comprising the DNA of claim 1.
23. A cell comprising the vector of claim 22.
24. The cell of claim 23, wherein said cell is a unicellular organism.
25. The cell of claim 23, wherein said cell is a yeast cell.
26. The cell of claim 23, wherein said cell is a mammalian cell.
27. The cell of claim 23, wherein said cell is a human cell.
28. The cell of claim 23, wherein said cell is a non-human mammalian embryonic blastocyst cell.
29. A transgenic non-human mammal produced by intrauterine implantation of said blastocyte comprising said cell of claim 28.
30. One or more progeny of said transgenic mammal of claim 29, wherein the DNA of said progeny comprises said DNA of claim 1, or a fragment thereof.
31. A method of directing expression of a polypeptide, said method comprising:
- a) providing a cell comprising the DNA of claim 13;
 - b) culturing said cell under conditions that allow for the expression of said polypeptide;
 - and
 - c) expressing said polypeptide-encoding sequence;
- thereby directing expression of said polypeptide.

32. The method of claim 31, wherein said cell is a renal cell.
33. A method of increasing transcription of a polypeptide-encoding sequence in tissue, said method comprising:
- a) providing in said tissue a cell comprising the DNA of claim 13;
 - b) culturing said cell under conditions that allow for the transcription of said polypeptide-encoding sequence; and
 - c) expressing said polypeptide-encoding sequence;
- thereby providing increased transcription of said polypeptide in said tissue.
34. A method for identifying a test compound that modulates expression from a *cis*-acting KIM-1 derived regulatory sequence, said method comprising:
- a) contacting said test compound and a reporter construct comprised of a reporter gene, operably linked to said DNA of claim 1; and
 - b) detecting the level of expression of said reporter gene;
- wherein a change in the level of expression relative to the level of expression in the absence of said test compound indicates that said test compound modulates the activity of said KIM promoter.
35. A method for delivering a therapeutic polypeptide to renal tissue of a subject, said method comprising:
- a) providing in said renal tissue a cell comprising the DNA of claim 13;
 - b) culturing said cell under conditions that allow for the expression of said polypeptide; and
 - c) expressing said polypeptide-encoding sequence;
- thereby delivering said therapeutic polypeptide to said renal tissue of said subject.
36. The method of claim 35, wherein said stimulus is injury.
37. The method of claim 36, wherein said injury is an ischemia-reperfusion injury.
38. The method of claim 36, wherein said injury is a nephrotoxic injury.

39. A method for treating or preventing renal tissue injury, the method comprising:

- a) providing a cell comprising the DNA of claim 13;
- b) culturing said cell under conditions that allow for the expression of a therapeutic polypeptide-encoding sequence;
- c) expressing said therapeutic polypeptide-encoding sequence; and
- d) contacting said tissue with said cell expressing said therapeutic polypeptide-encoding sequence;

thereby treating or preventing renal tissue injury.

40. A method for increasing transcription of a nucleic acid in a subject, the method comprising administering to said subject the DNA of claim 4, wherein said operably linked DNA is expressed in an amount sufficient to result in increased transcription of said operably linked nucleic acid.

41. A method for treating or preventing renal tissue injury in a subject, the method comprising administering to said subject in need thereof the DNA of claim 13, wherein said operably linked DNA is expressed in an amount sufficient to treat or prevent renal tissue injury in said subject.

1 GATCATACAA ACATGCTGTT ATTTTATCA CTTAAAAAA AAACACCCAG
51 GATTTTCTCC TTCCATTTTT GCAAACTTT TATTTTTTTT TTGGAAGATG
101 GGGACTCACT CTGTCACTCA GGCTGGAATG CAGTAGTACT ACCATATCTC
151 ACTGCAGCCT CAAACTCCTG GGCTCAAGTG ATCCCTCCCG CTTAGCCTCC
201 CAAATGGCTG GTACTATAGG CACTCAAGTC CAACTGCTTT TCTCCATGCA
251 AACTCCTTGA AAGTGTTTCC TGTATTCAAT TATCTCCTGA TTTTCCTTCT
301 TGTAACCTTT TTA CTG CAGT ATAAAGTACT GGGGCTCACT GATAATCTCC
351 AGCTTGCTCA GTCTATGACA AATCTTATTC CTTTCCTTTG CAGCATTGTA
401 CTCATGATTG CTGCCTGTTT TTTGATGCGT TTGCTTCACT TGGCTTCTAG
451 GACCTTTTTG CTTTTTCTCT TACCTCCTTG GGCTGCTTCC ATTTCTGTAT
501 TGGTGCCTCT TCCACCTCAG CATTTTTTTTT TTTTTTTTTT TTTAAGACGG
551 AGTCTCGCTC TCTCGCCCAG GCTGGAGTGC AGTGGTGCGA TCTCGGCTCA
601 CTGCAAGCTC CGCCTCCCAG GTTCACGCCA TTCTCCTGCC TCAGCCTCCT
651 GAGTAGCTGG GACTATAGGC GCCCGCCACC ACGCCCGGCT AATTTCCACC
701 TCAGCTTTAA CAAATTTTTT TAAATTAAT TAATTTTTTT TTTTGAGACG
751 GAGTCTTGCT CTGTCACTCA AGCTGGAGTG CAGTGGCATG ATCTCGGCTC
801 ACTGCGACCT CTGCCTCCCA GGTTCAAGCA ATTCTCCTGC CTCAGCCTCC
851 TGAGTAGCTG GGATTACAGG CATGCGCCAT CACACCCGGC CAATTTTTGT
901 GTTTTTAGTA GAGACGGGGT TTCACCATGT TGGCCAGGCT GGCCTGGAAC
951 TCCTGACCTC AAGTGATCAG CCTGCCTTGG TCTCCTAAAG TGCTAAGACT
1001 GCAGGTGTGA GTCGCCACAC CCGGCCTTAA AATTTATTCT TATGTAGAGA
1051 TGGTGTTC A CATGTTGGC CAGGCTGACC TGGAACCTCCT GACCTTAAGT
1101 TATCAGCCTG CCCCAGTCTC CCAAAGTGTT GGGATTACCT GCATGAGTCA
1151 ACATGCTTGT CCCCATTTTA ATCTTTTGAT GCTGGAAGGC CCCAGGACCT

Fig. 1A

1201 AGTCCTTAGC ATCAGGCATT CCTTTGAATC TCATCCTTTG AATTCCTACC
1251 TCATTTCAGGC TCCTGGCTTT AAAATACCAT TTTTTTTTTT TTGAGGCGGA
1301 GTCTCGCTCT GTCGCGCAGT GCGCGATCT CAGCTCACTG CAAGCTCCGC
1351 CTCCCAGGTT CACACCATTG TCCTGCCTCA GCCTCCCGAG TAGCTGGGAC
1401 TACAGGCACC TGCCACCACG CCTGGCTAAT TTTTGTATT TTCAGTAGAG
1451 ACGGGGTTTC ATCGTGTTAC CCAGCACAGT CTCGATCTCG TGATCCGCCC
1501 ACCTCGGCCT CCCAAAGTGC TGGGATTACA GCGTGAGCC ACCGCACCCA
1551 GCCAATACCA TTTCTAAGCC AGTAACTTGT AACTGTATCT TTAGCTCAGA
1601 CCTCCCTCCT GAACTCCAGC AGTCTCCACA CAGGTCTAAG ACATGTCAA
1651 CTCAACATAC TTAAAACCCT GAATATTTCC TCTAAAACCT GTGGTCATGC
1701 AGGTTTTTGT TTTTGTGTTT TTGTTTTTTT TGAGATGGAG TCTTGCTCTG
1751 TTGCCCAGAC TAGAGTGCAG TGTACGATC TTGGCTCACT CCAACCTCTG
1801 CCTCCTGGGT TCAAGCAATT CTCCTGGCTC AGCCTCCTGA GTAGCTCAGA
1851 TTACAGGCAC CCACGACCAT GCCTGGCTAA ATTTTGTAT TTTTAGTAGA
1901 GACAGGGTTT TGCCATGTTG GCCAGGTTGG TCTTGAAGTC CTGACCTCAG
1951 GTGATCCACC TGCCTTGGCC TCCAAGGTG TTAGGATTAC AGGTGTGAGC
2001 CACTGAGCCC AGCCTTTGCA GCTCTCCTTG TCTTAATTGG CTGGAACCTC
2051 CAGCTCTTCC CGTGGCTCAG GCCGAAATCC TTGGAGTCAT CTTAGGCCCT
2101 TTCTCCTCAT ATCCTACAGG AAATCCTGTT TGCTCCACCT TCTCCACCTC
2151 CTTGGCTCAA GCCATTCTCC TGCCTCAGCC TCTTTAGTAG CTGGGACTAC
2201 AAGTTGCATG CCAGCATGCC TGGCTAATTT TTCTTTTTCT TTCTTTTTTT
2251 TTTTTTTTTG TAGAGACAGG GTCTCACTAT GTTGCCCTGA GCTCCTGGGC
2301 TCAAGCAGTC CTCCCGCCTT GGCCTCCCAA AGTCCAGGGA TTACAGCTGT

Fig. 1B

2351 GAGCCATCAC ATCTGGCTAC TCTAGGTTGA GTGAGGAAAG TTCATTGACC
2401 ACTTCCACTG CTAACCCATC TCTTCTGGAA TCTTTCCATA GTCTCCTGAC
2451 AGGTCTTCCT GCTTCTCAAT CTAGCAACCA CAGTGGTCCT TCTCAAAGGA
2501 AGTTAGATAC TGTACCCTA TGCCCTTGCA GTGGTGCTTC TTTTCATGTG
2551 GGGTGAAAGC CTATGTCCTC AGAATATGGC TCCTAAGCCC CATGTGTCTG
2601 TCCTCTGCCC TCACTCCTCT GTGATCCCTG TCCCTCGCTC TGTTCAGTC
2651 ACGCTGGCCT CTCTTGCCCT GTAAACACAC CAGGCACCCT CCTGCCTTAG
2701 GGCCTTTGCC CTTCTTGTCT GTCTCCATGG AAAGCGTTTG CTGTCTTGGC
2751 TAACTTCCTT GTCCTTTGTC TTAGTTCAAA TAATCACCTT CTTGGTGAAA
2801 GTAATAGAGA CTATTCAAAC CTGACCACCT TGTTTAAAT TGCAACTCAG
2851 TGCCTCCTCA ACCCTCCACT CCCAACCACC TTCACCCTGC TCTTGTGTAT
2901 CCTTTTGCCCT TTTTTCATT AGCATTCTC AACTTGTAAT ATGCTGATAA
2951 ATTACATTTT AGTGATGTTT TAAAAATCTG TATATTTATT TTTTCAGTTAA
3001 AAGTTAGTTA CATGAGGCCA GGAGTGGTGC TCACGCCTAT AATCCCAGCA
3051 CTTTGGGAGG CCAAGGCGGG CAGATCACTT GAGGTCAGGA GTTCGTGACC
3101 AGCCTAACCA ACATGGTGAA ACCCCGTCTC TGCTAAAATT AAAAAATTA
3151 GCCGGTGTGG TGATGCATGC CTGTAATCCC AGCTTCTTGG GAGGCTGAGG
3201 TAGGAGAATC GCTTGAACCC AGGAGGCAGA GTTTGCAGTG AGCTGAGATC
3251 GTGCCATTGC CCTCCAGCCT GGGCAAAAAA AGCGAAGCTC CATCTCAAAA
3301 AAAAAAAAAA AAATGTAAGT TACATGAGGC CAGGGGTCTT TGGTTCATTG
3351 GTACATTCCA GATGAATAGG ATCATTCTA ACATATCGCA GATCATCAAC
3401 AAATAATTGT TAAATGAGTA CACTTTTGGT ATTTTATAT ATTTTCTTTC
3451 TTTCTTTCTT TCTTCTTTT TTTTTTTGAG ACAGAATCTC GCTCTGTCAC
3501 CCAGGCTGGA GTGCAGTGGT GTGTGATCTC AGCTCACTGC AACCTCCACC

Fig. 1C

3551 TCCCAGGTTC AAGCGATTCT CTTGCCTCAG CCTCCCTAGT ATCTGAGACT
3601 ACAGGCACGC GCCACCACGC CTGGCTAATT TTTGTAGTTT TAGTAGAGAC
3651 AGGGGTTTGC CATATTGGCC AGGCTGGTCT TGAACCTCCTA ACCTCAAGTG
3701 ATCCTCCTGC CTTGGCCTCC CAAAGTGCTG GGATTACAGG TGTGAGCCAC
3751 CATACTTGGG CTTTTATGTA TTTTCTATGG TAAACATAGG TGGTACCCTG
3801 TAATTTTTAT ATCTTTGTAA AAGATATAAA AAAAAGAAGC ATTATATTAC
3851 TTGTTATGAA ATCAGAGGAG TAAGTGAAGG AAAATAACTA GCTTAGGGCA
3901 GTGGGCAGGG CAGGAAGAGA ACTGAAAGGT AGGAAGACAG TTTTGGAGGG
3951 AATTGCAGAA GTCTGGATTA TAGAGGCCTA ATATAAAGTG ATGGGGATGA
4001 GGGAGAGACT GACAGGTACA ATGATGTGGA GTTGGTGAGT CCCTAGTTGT
4051 GGAGGGGGCC TAAGAAGATC TTGCTGTGGT GAAAGCATGG GGAATATGAA
4101 CAGCTGAACT GTTTTGCAGG AGGCTGGAGC TGGAGGTACG ATGTGCGCTG
4151 AGATAGCAGG GAAGTAAGTG GTGATTGCAA GAAAGAACAG TGAATTATTT
4201 TCTTTTCTGA ATTCTTTCTT TTTTTTGAGA CAGGGTGTCA ATCTGTTGTC
4251 CAGGCTGGAG TGCAGTGGCA CGATCTCAGC TCACTGCAAC CTCCACCTCC
4301 CGGGTTTCGAG CAATTCTCCT GCCTCAGCCT CCAAGTAGC TGGGATTACA
4351 GGCACCCACC ACCGTGCCCCG GCCCATGTTC TGAATCATTT CAATTCCTG
4401 CCGTTAATCT TGGTTTATAC AGATGCAGCT CCCTAGTGAG CAGCTGGAAA
4451 TTCAGCTTTG GTGCCCAAGT ATTGTCACTT CCAGCTTTAC CCTACAACCTG
4501 GGATGCATCC TTCAGGGGGG TCATGAAGTT TGCCCTAAAG AGTAGTGATC
4551 CCTGGAGGTT GTATAGCTCA TTAAAAAAT CCACTGTGCT ATATTGTTTG
4601 GGAGTCTTTA GAACACAGGC GTCTCTCATG GGAGATGGTC CTGTGTCAGA
4651 AAATTCAACC CTATGGAATT GTACAGTTAT GTAACATCTC AGAGCCTTGG

Fig. 1D

4701 CTCCACATCC CTGTCCTGGC TCTCTCTGGC TCATCATTTT CTCCAGTTGA
4751 AACACCCTCC ACCCATTTCT CTCACATGTC ACTTTTTTAAG AAATTCTTCC
4801 CACCCCCCAC ATTCCGTCAT CAAAATGAAT GGTCTTTCCC TATGGGTTTG
4851 TGTTCCTCATT TGTTCATCTA TTCAATTAAT AACTTTTTTT TTTTGTAGAA
4901 GTCTCACTCT GTGGCCCAGG CCAGAGTGCA GTGGCATGAT CTCCGCTCAG
4951 GGTAAATTCT GCCTCCCGGG TTCAGGCGAT TCTCTTGCCT CAGCCTCCTG
5001 AGTAGCTGGG ATTACAGGCA CCCGCCACCA CGCCTGGCTA ATTTTGTGAT
5051 TTTTGGTAGA GTTGGGTTTC ACCATGTTGG CCAGGCTGGT TTGGAACCCC
5101 TGACCTCAAG TGATCCTCCC ACCTCGGCCT CCTTTGGATT ACAGGTGTGA
5151 GCAACCATGC CTGGCTTCAA CACTTAAATT GCCTTAAAGG AGTTTATGGT
5201 CTGGAGTTGG GTGCCACACA ACACAGTCAC TATGTGTGAC AATTTAAATT
5251 TTATTTTTTT GTTTTAAATT AATTTATTTT TTTGAAAGCT CTGTCATCTA
5301 AGGCTTGAGT GCAGTGGTGC CATCTCAACT CCCCGAAGAC TGTCTCCTGG
5351 GCTCAAGCAA TCTGAAATTT TAATTAAAT GAAATTAAAT AAAAATTTTT
5401 AGGCCAGGCA TGGCGGCTCA CACCTGTAAT TCCAGCACTT TTGGAAGTTG
5451 AGATGAGCGT ATCACTTGAG GCCAGGAGTT CCAGCCCAGC CTGGCCAACA
5501 TGGTGAAACT CCACCTCTGC TAAAAATACA AAAATTAGCC AGGCATGGTG
5551 GCGCGTGTCT GTAGTCCCAG CTACTCAGGA GACTGTGGCA AGAGAATCAC
5601 TTAAACCCAG GAGATGGAGG TTGCACTGAG CTGAGATTGT GACACTGCAC
5651 TCCAGCCTGG GTGACAGAGT CAGGCTCTGT CTTGGAAAAA AAAAAATTA
5701 AAAATGCCTT GGTTGCCTTA GCCACATTTT AAGTGCTCAA TAGTCATATG
5751 TGGCTAGTGG CTGCTGTAGT GCACGACACT CACACAGAAT AACTCTGTAA
5801 CCAATATTCT ACTGGAGACA GAATCGATCC TATGGAATTC AAATTCAAAT
5851 CCTATGGAAT TGTACAGTTA TGTAACATCT CAGAGCACTG GCTCCACATC

Fig. 1E

5901 CCTGTCTTGG CTCTCTGTGG CTCATCAGTT CCAGAATAAC TCCGTTACCA
5951 GAATAACTCC ATTACTAAAA TTCTACCGGG CAGCACTCTA TAGGAGGGAA
6001 TAGAGACAGA CACCACATAT ATTGCACACA CAGATAAAAT GGATTAAGGA
6051 AAACAAGATA ATAATAGTGA GAGGGACTGG TTGGCTACTT TAGATTGAAG
6101 GACCTGTGAA AAATGTCCAG GGAGGTCATA TTTAAGCCGG GATAAAAATG
6151 AAAAGGAAAA AAGTGAAAAT GGTGGGGCTG GGGAGCTAGA TGGAGAACAC
6201 AGCCACGGAA AAGGCCTTAG GGTGAGGCA AGTTGGAAAG AAAGCTCTAG
6251 TAGCTGGGGC TGAGTCAGCA GGGGAGAGAG TGGTAGAAGA AATCTATGGG
6301 GTAGGTCAGG GCCAGACCAC CAGGGCTTCA GTAATTTGAG TAAAGATTTA
6351 GGAATTATTA TTATTATTAT TATTATTATT TTTCTGAGAG AGTTATGAGA
6401 GGGTTATAAG TGGGGGAATG ATGTAGTCTG ATTATATATT TACCTTTACC
6451 TCACTTATCC TGATTTTATT AGTTGCTTAC TTACCCATGT CCCTGCCCCA
6501 TTGCACAAGT CTGGATTTTT GACGTCCCTA GTATATTGAG TCATGTCCCA
6551 TCAGCTCAAT ATGTTAGTAA TAACTGGTTG AATTGAATTA GCTTTTTTTT
6601 TTCAATCTTT TTTTCCTTAA GAAACAGGGT CTTGCTCTGT CACCCCGGCT
6651 GGTGTGCAGT GGCACAATCA TAGCCTCAA CTGCTGGGCT CAAGCAACCC
6701 TCCTGCCTCA GCCTCCTGAG TAGCTGGGAC TACGGTCAGG TACACAAGGC
6751 CTGACTATAT TTTTGTTCG TTTTTTTGCA GAGAGGGAGT CTTGCTATGT
6801 TGCCCAGGTT GGTCTCAAAC TCCTTACCTC AGGTGATCCA CTTGCCTTGG
6851 CCTCCCAAAG TGTGCGGATT ACAGGCGTGA GCCACTGTGC CTGGCAAGAA
6901 ATGAATTTTT ATTTTATTTT TTGAGATGGA GTTTTGTCT TGTGTCCAG
6951 GCTAGAGTGC AATGGCTTGA TCTCGGCTCA CTGCAACCTC CACCTTCCAG
7001 GTTCAAGCAA TTCTTCTACC TCAGCCTCCT AAGTAGCTGG GATTACAGGC

Fig. 1F

7051 GCCCGCCACC ACCCCCAGCT AATTTTTGTA TTTTATAGTAG AGTCGGGGTT
7101 TCACCGTGTT AGCCAGGCTG GTCTTGAAC CCGACCTCA GGTGACTGGC
7151 CTACTCGGCC TCCCAAAGTG CTGGGGTTAC AGGCACGAGC CACCATGCCC
7201 GGTCAAGAAA TGAATTTTTA AACGCTGCCA TACAAAACAC TATGCTGAGA
7251 TCATCCACTT CCCCATGAAC CCTGTCATGA GCTGCAAGAT ACAGACCACC
7301 ACTGCCTCCT TGAAGTTAC TGAATTCTTA GACCAGAAGA GGAGTTAATG
7351 AAGTACTAGG CAAGCTTACT CATGTTTGTA TGGTTTAATG ATTAACAGCA
7401 GAAGTCAACA GCCCGATTTA ACGCATGTGG GTGCTTGACA CAGAGCCTGC
7451 TATATAGTAT TCTCCAAAAA CCTCAGCTAG TGCTATTACT GCATATGATG
7501 TAGGTTTAGT TTTCCAAGTT CTTCCGTGGC CCTTTTTGCT TATTATATCA
7551 ATCCTTGGTG GGAGATAGAG GAAGCATTTT TAGTGCTATT TTACAACTGA
7601 GGAAATAGAG GTTTGAAGAG AACTCAGGAA CTCTCAGGGT TACCCAGCAT
7651 TGTGAGTGAC AGAGCCTGGA TCTGAACGTA AGTCTGCTCC AGACTTCTGT
7701 TTCCTGAAGC ATTCTCTTGA AGTCCCTTGG TAAGGAGGTG TAGTCTGAAG
7751 CATGTTGTAC AGGAGCATGA AAGGTTAGGC ACAGTGATTC ACATTCACTC
7801 TCAATTTCTC TTGCTAATGG CAAACTTGGC AATATGACTG TTAAGGCTAG
7851 GGATAAGTCG TTGTGGCCAC TGAGTAGGAA AAGCTCCACG TCCACCAGAG
7901 GCCCAGTTTA CTCTGAAAAG CAAGTGCATC TCTGCCACTG GAAGGCTGGC
7951 ATTTGCTCTC GTGCTGCCAT TGAGCCACGC TGTTCTCTG CTTCCAGTTT
8001 CCTTTTCTTT TCTTTTTTTT TGTTTTGTTT TTTGAGACGG AGTCTTGCTC
8051 TGTCGCCCAG GCTGGAGTGC AGTGGCGCGA TCTCGGCTCA CCGCAAGCTC
8101 CGCCTCCCGC GGGTTCACGC CATTCTCCTG CCTCAGCCTC CCGAGTAGCT
8151 GGGACTACAG GCGCCAGTGA CCACGCCCGG CTAATTTTTT GTATTTTTAG
8201 TAGAGACGGG GTTTCACCCT TTTAGCCAGG ATGGTCTCGA TCTCCTGACT

Fig. 1G

8251 TCGTGATCTG CCCGCCTTGG CCTCCCAAAG TGCTAGGATT ACAGGTTTGA
8301 GCCACCGCGC CCGGCCCTGT TTCCTTTTTG TTTGTTCCCC TGATACCCTG
8351 TATCAGGACC AGGAGTCAGT TTGGCGGTTA TGTGTGGGGA AGAAGCTGGG
8401 AAGTCAGGGG CTGTTTCTGT GGACAGCTTT CCCTGTCCTT TGAAGGCAC
8451 AGAGCTCTCA GCTGCAGGGA ACTAACAGAG CTCTGAAGCC GTTATATGTG
8501 GTCTTCTCTC ATTTCCAGCA GAGCAGGCTC ATATGAATCA ACCAACTGGG
8551 TGAAAAGATA AGTTGCAATC TGAGATTTAA GACTTGATCA GATACCATCT
8601 GGTGGAGGGT ACCAACCAGC CTGTCTGCTC ATTTTCCTTC AGGCTGATCC
8651 CATAATGCAT CCTCAAGTGG TCATCTTAAG CCTCATCCTA CATCTGGCAG
8701 GTAAGTGAGT AGGTGCCCTG GCGGGAAGA AGGGAGTAGA GGGGGGTTAG
8751 AAGCCAGAGA ATGGGGTAGG GGAAGGGGAG GGGATGGTGG TGGTGGATTA
8801 ATGTAGATGT TCTTTGGGTA CCGTTGTATG GCTATGAGTT AACTAGTGAG
8851 CAGGACCAGA ATAAAGTTTT AGGCCAAAGA AATTGCTTAA CTGCTGTGAA
8901 TTACAACATT CATGGCTAAA TGAACAAGGC AAG

Fig. 1H

human KIM-1 genomic clone (MZ007):

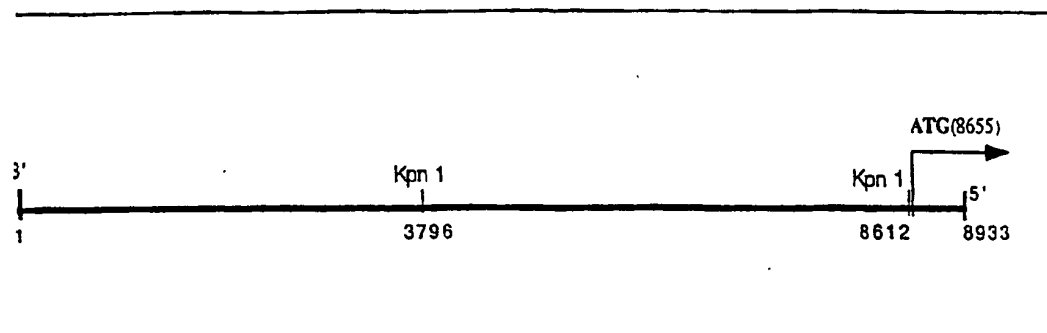


Figure 2A

human KIM-1 5' regulatory Luciferase constructs: *

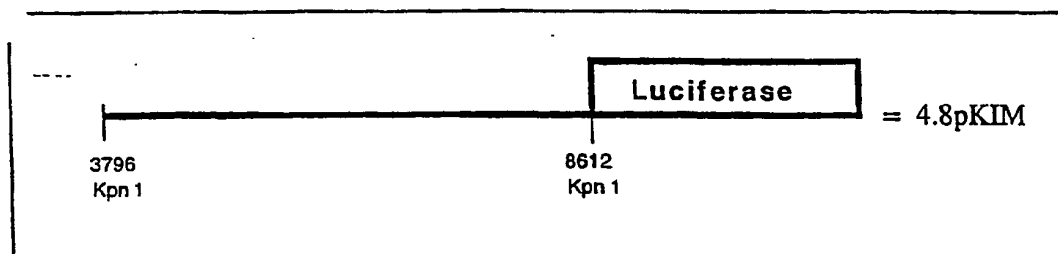


Figure 2B

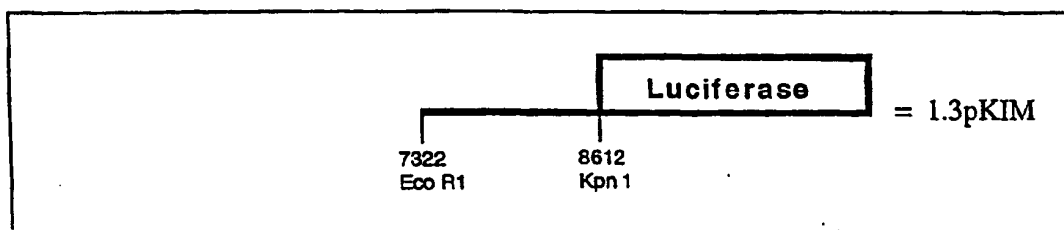


Figure 2C

human KIM-1 5' regulatory Luciferase constructs, continued:

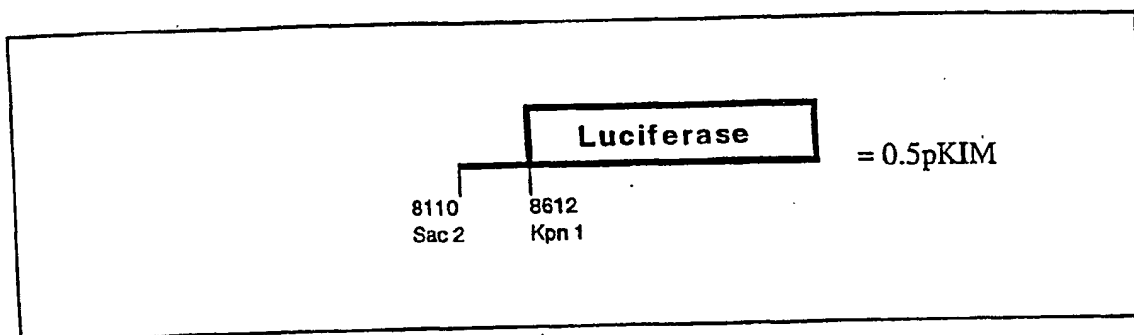
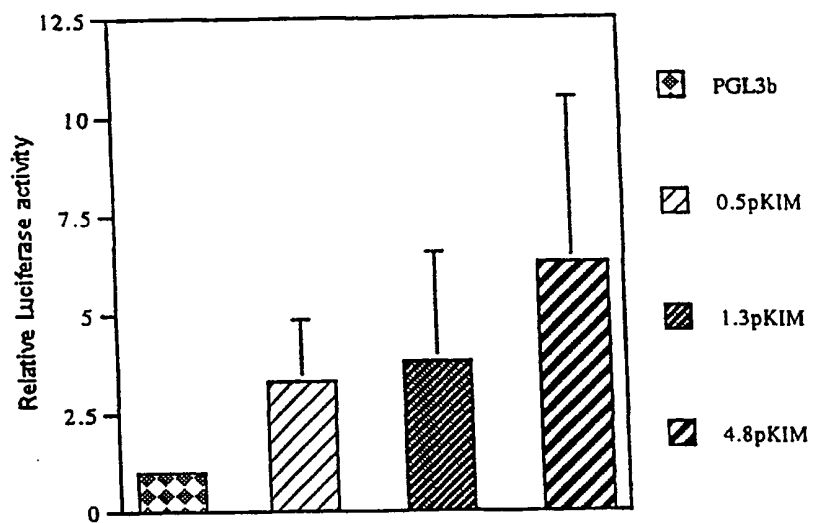


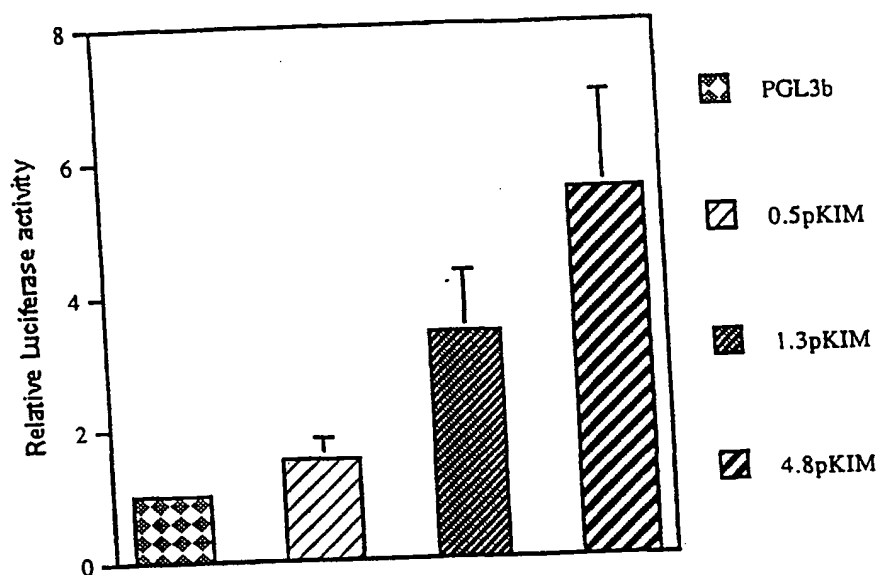
Figure 2D

* All constructs are in PGL₃b.

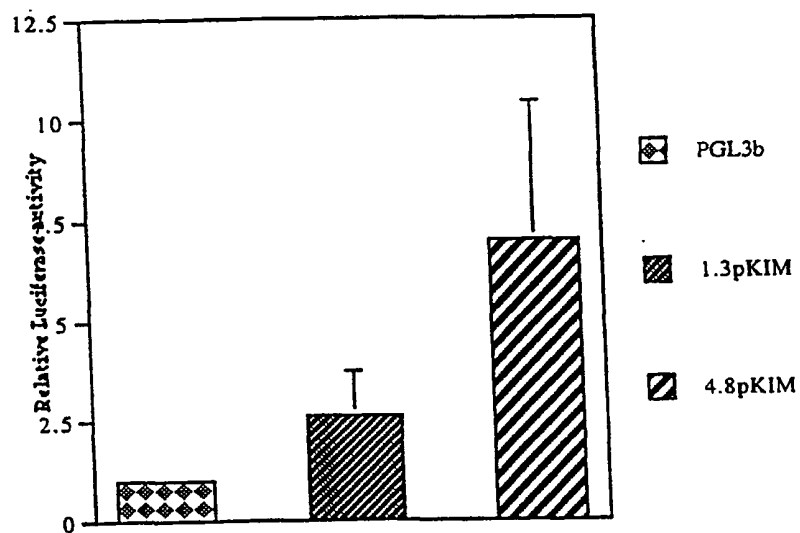
Transient transfection of pKIM-1 deletion constructs in COS cells



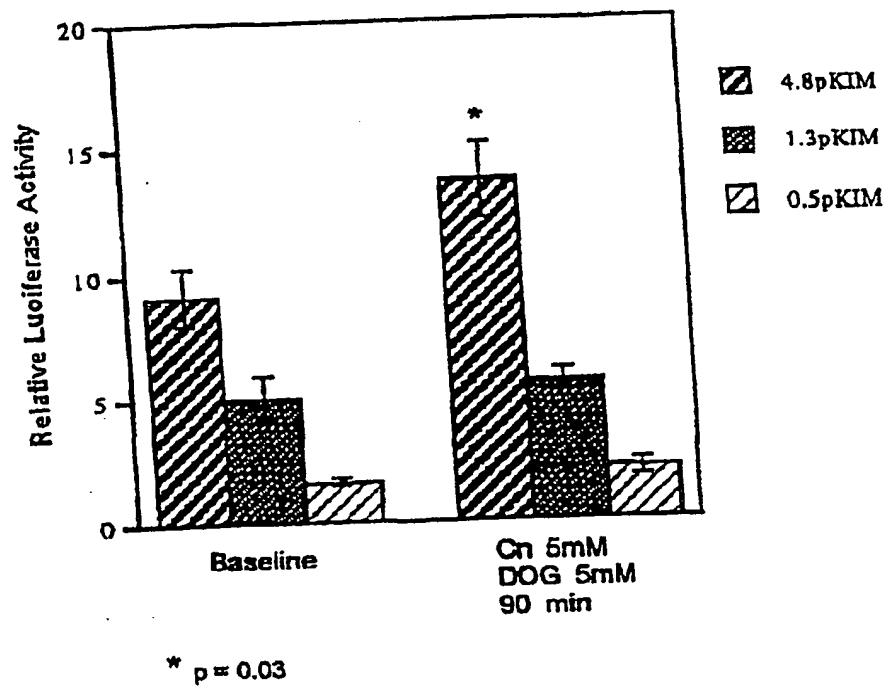
Transient transfection of pKIM-1 deletion constructs in LLC PK cells



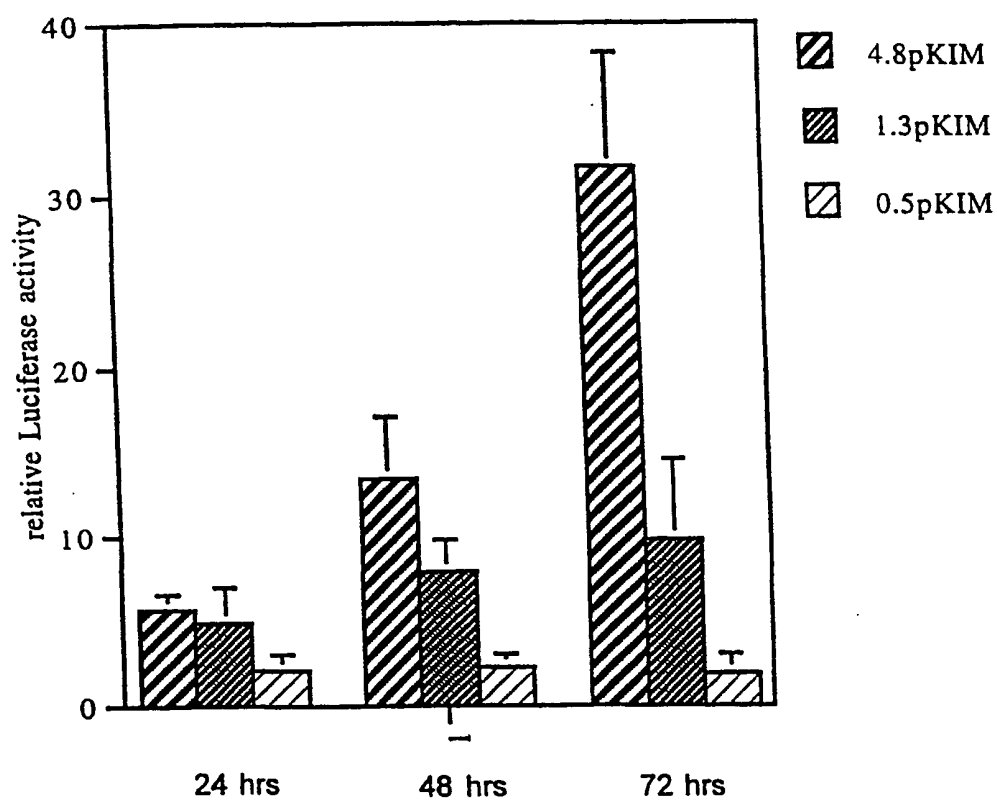
Transient transfection of pKIM-1 deletion constructs in MDCK cells



Induction of pKIM-1 deletion constructs by chemical anoxia



MDCK cells at various timepoints after transfection
with KIM-ptomoter deletion constructs (n=4)



SEQUENCE LISTING

<110> Biogen, Inc.

Sanicola-Nadel, Michele

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<120> RENAL REGULATORY ELEMENTS AND METHODS OF USE THEREOF

<130> 00689-502-061 (BGN-2)

<140> Filed herewith

<141> 2001-06-15

<150> USSN 60/212,131

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HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
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IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
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Published:
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(54) Title: RENAL REGULATORY ELEMENTS AND METHODS OF USE THEREOF

(57) Abstract: Disclosed are *cis*-acting regulatory elements from a KIM-1 gene. The elements can be used to direct the expression of operably linked sequences in renal tissue.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/19295

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/00 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL-EBI 'Online! 27 March 2000 (2000-03-27) DOE JOINT GENOME INSTITUTE STANFORD HUMAN GENOME CENTER.: "Homo sapiens chromosome 5 clone CTC-332D4, complete sequence." retrieved from EMBL Database accession no. AC026777 XP002207936 abstract</p> <p style="text-align: center;">--- -/--</p>	1-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *G* document member of the same patent family

Date of the actual completion of the international search

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Paresce, D

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/19295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL-EBI 'Online! 23 November 1998 (1998-11-23) ADAMS M.D., ET. AL.: "CITBI-E1-2517G14.TF CITBI-E1 Homo sapiens genomic clone 2517G14" retrieved from EMBL Database accession no. AQ277590 XP002207937 abstract</p> <p>---</p>	1-11
X	<p>DATABASE EMBL-EBI 'Online! 27 April 1998 (1998-04-27) PHILLIPS S.: "Human DNA sequence from clone 109F14 on chromosome 6p21.2-21.3." retrieved from EMBL Database accession no. AL022721 XP002207938 abstract</p> <p>---</p>	1-11
Y	<p>ICHIMURA T ET AL: "Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 273, no. 7, 13 February 1998 (1998-02-13), pages 4135-4142, XP002079926 ISSN: 0021-9258 cited in the application see p. 4135-6, fig. 1</p> <p>---</p>	1-41
Y	<p>WO 97 44460 A (HESSION CATHERINE A ; ICHIMURA TAKAHARU (US); WEI HENRY (US); BIOGE) 27 November 1997 (1997-11-27) cited in the application see p. 2-4, p.15-30</p> <p>---</p>	1,4-41
Y	<p>WO 98 20110 A (KRISSANSEN GEOFFREY W ; UNIV AUCKLAND (NZ); LEUNG EUPHEMIA YEE FUN) 14 May 1998 (1998-05-14) see abstract, claim 1 (f)</p> <p>---</p>	1-41
Y	<p>FAURE C ET AL: "DIFFERENTIALY EXPRESSED GENES IN ISCHEMIC ACUTE RENAL FAILURE" MOLECULAR BIOLOGY OF THE CELL, BETHESDA, MD, US, vol. 9, 1998, page 473A XP000953127 ISSN: 1059-1524 the whole document</p> <p>---</p>	1-41

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/19295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ROSENBERG ET AL: "Differential gene expression in the recovery from ischemic renal injury"</p> <p>KIDNEY INTERNATIONAL, NEW YORK, NY, US, vol. 39, no. 6, June 1991 (1991-06), pages 1156-1161, XP000953186</p> <p>ISSN: 0085-2538</p> <p>see abstract</p> <p>----</p>	1-41
A	<p>PADANILAM ET AL: "Molecular mechanisms of cell death and regeneration in acute ischemic renal injury"</p> <p>CURRENT OPINION IN NEPHROLOGY AND HYPERTENSION, RAPID SCIENCE, LONDON, GB, vol. 8, no. 1, 1 January 1999 (1999-01-01), pages 15-19, XP000953223</p> <p>ISSN: 1062-4821</p> <p>the whole document</p> <p>-----</p>	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/19295

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 1-41 partially
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 34 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claims 31-33, 35-41 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 1-41 partially

Present claims 1-41 relate to an extremely large number of possible compounds/products. The term "comprising" and the phrase "KIM-1 derived regulatory sequence" in claim 1 of the present application are so vague and unclear that the scope of the claim includes any possible isolated DNA. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out only for those parts of the claims which appear to be supported and disclosed, namely the DNA sequences shown in SEQ ID NO: 3 and SEQ ID NO: 2 of the present application (claims 2-3) as well as methods involving the use of said DNA sequences (all other claims searched partially).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/19295

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9744460	A	27-11-1997	
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